PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JVK-ChP-V127	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/50402	International filing date *(day/month/year)* 11.09.2003	Priority date *(day/month/year)* 12.09.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/68		
Applicant VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR ...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the opinion
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☐ Lack of unity of invention
   V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.03.2004	09.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Weijland, A Telephone No. +49 89 2399-7490

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/50402

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-36 as originally filed

Claims, Numbers

1-12 as originally filed

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

   see separate sheet

The following documents (D) are referred to in this report; the numbering will be adhered to the rest of the procedure:

D1: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 289, 2001, Pages 161-166.
D2: JOURNAL OF ENDOCRINOLOGY 168, 2001, Pages 283-296.

1. D1 is added by the own knowledge of the examiner, a copy will be attached to this opinion.

2. Novelty (Article 33(2) PCT)

The subject matter of claims 1-12 is anticipated by D1 and D2 and is therefore not novel.

D1 (abstract; page 162, left column, last paragraph; right column, first paragraph) describes recombinant PCP4MO ("target molecule" according to claim 1) produced in E.coli ("complex mixture" according to claim 3) as a glutathione S-transferase (GST) fusion protein. Moreoever a tobacco etch virus (TEV) protease cleavage site was introduced. A two step purification protocol was developed to obtain large quantities of rePCP4MO. GST-PCP4MO was cleaved either in eluent or on column by factor Xa or His-tagged protease. In eluent, glutathione was removed by factor Xa ("enzymatically" according to claim 1) and GST and uncleaved fusion protein was removed by rechromatography with glutathione-sepharose ("compound-target complex", "separating...via chromatography", "first isotpoe...second isotope" according to claims 1 and 9). The resulting rePCP4MO was identified as apoprotein and the catalytic activity determined.

D2 (abstract; page 286, right column, second paragraph; pages 288-290) describes the IGFBP-4 protein fused to hexahistidine and which can be cleaved with a TEV protease site. The protein is produced in E.coli and purified using nickel-chelating affinity chromatography. After cleavage, a second nickel chelating step ("compound-target complex", "chromatography", "first isotope...second isotope" according to claims 1 and 9) was carried out and final purification by reverse HPLC. The purified protein was analysed by mass spectrometry.